Molecular Identification of Korean Mountain Ginseng Using an Amplification Refractory Mutation System (ARMS)

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Abstract: Expensive herbs such as ginseng are always a possible target for fraudulent labeling. New mountain ginseng strains have occasionally been found deep within mountain areas and commercially traded at exorbitant prices. However, until now, no scientific basis has existed to distinguish such ginseng from commonly cultivated ginseng species other than by virtue of being found within deep mountain areas. Polymerase chain reaction (PCR) analysis of the internal transcribed spacer has been shown to be an appropriate method for the identification of the most popular species (*Panax ginseng*) in the *Panax* genus. A single nucleotide polymorphism (SNP) has been identified between three newly found mountain ginseng (KGD4, KGD5, and KW1) and already established *Panax* species. Specific PCR primers were designed from this SNP site within the sequence data and used to detect the mountain ginseng strains via multiplex PCR. The established multiplex-PCR method for the simultaneous detection of newly found mountain ginseng strains, Korean ginseng, and foreign ginseng in a single reaction was determined to be effective. This study is the first report of scientific discrimination of “mountain ginsengs” and describes an effective method of identification for fraud prevention and for uncovering the possible presence of other, cheaper ginseng species on the market.

Key words: amplification refractory mutation system (ARMS)-PCR, multiplex PCR, *Panax ginseng*, single nucleotide polymorphism (SNP)

INTRODUCTION

Ginseng (*Panax ginseng*) is one of the most important medicinal plants in East Asia, throughout which almost every species of the genus has been employed as a source of medicine. Ginseng literally means “the essence of the human” [1] and is also known as the “king of herbs.” China and Korea have utilized this plant for more than 2000 years as a tonic, a stimulant, and an agent to foster fatigue and stress-resistance [2]. Recently, the pharmaceutical effects of ginseng roots have been demonstrated by a host of studies and ginseng has become a world-renowned medicinal plant. The active constituents contained in most ginseng species include ginsenosides, polysaccharides, peptides, polyacetylenic alcohols, and fatty acids [3]. Ginseng is known to improve antibody-dependent cell cytotoxicity [4], reduce lung pathology [5], bolster learning in mice [6], potentiate vaccination against the common cold and/or influenza [7], inhibit the development of reverse tolerance to morphine [8], prevent injuries from oxygen free radicals [9], exert anti-stress effects [10], inhibit mutagenesis [11], potentiate the generation of nerve fiber [12], and exhibit antiaging properties [13].

Ginseng belongs to the genus *Panax*, which consists of 17 species, only some of which have been widely cultivated due to their profound medicinal effects. These are *P. ginseng* (Korean ginseng), *P. japonicus* (Japanese ginseng), *P. notoginseng* (Chinese ginseng), and *P. quinquefolius* (American ginseng) [10]. Normally, cultivated ginseng is systematically farmed on open land with proper control of sunlight by shielding (canopy) that helps to reduce 1/8-1/13 of total sunlight and is harvested after a 5-6 year cultivation period. Occasionally, however, indigenous mountain ginseng has been discovered and traded at very high prices. Mountain ginseng, found deep within mountain areas at altitudes between 800 and 1,500 m, is much slower in growth and more sensitive to environmental changes than cultivated varieties and exhibits a preference...
for areas with fluctuating daily temperatures and minimal exposure to direct sunlight. The hardiness of mountain ginseng has been commonly thought to result in more pharmaceutically active roots and some mysterious effects on the human body.

Traditionally, authentication of ginseng has relied upon morphological and histological inspections, but in many cases, such approaches are far from reliable. For example, most ginseng roots are extremely morphologically similar, thus rendering their differentiation almost impossible for the layperson. The illegal practice of disguising common cheap ginseng as mountain ginseng has become a more prevalent problem in recent years. Determining the source of a plant from its morphology is frequently difficult, as the majority of ginseng products are sold in the form of powder or shredded slices.

In eukaryotes, two internal transcribed spacers (ITS1 and ITS2) that flank the 5.8S rDNA region have been identified, which are co-transcribed with the 5.8S rDNA, but not translated [14]. The ITS sequences are highly conserved throughout plant species but are suitable markers for the interspecies variation and intraspecies conservation. Thus, ITS sequences are suitable targets for investigations of plant phylogenetic relationships within the same genus and species [15-18]. The phylogenetic relationship of the Panax genus has been more recently studies and so the relationship of any new strains found within Panax could be better positioned.

In this study, we found three new mountain ginseng strains through the analysis of the ITS regions. The ITS sequences were different from those of already established Korean ginseng and other foreign ginseng. This report presents a more reproducible and robust approach for the authentication and differentiation of a mountain ginseng from already established ginseng species via single nucleotide polymorphism (SNP) analysis.

**MATERIALS AND METHODS**

**Ginseng materials**

Actively growing ginseng roots were collected from Korean Ginseng Center and Ginseng Genetic Resource Bank, Kyung Hee University, Republic of Korea. Five samples of ginseng were selected: Panax ginseng Ja-Kyung variety, P. ginseng Chun-Poong cultivar, P. japonicus, P. notoginseng, and P. quinquefolius. Three samples of mountain ginseng roots were obtained from Odaesan (Mt. Odae) by “mountain ginseng diggers,” also known as simmani in the Korean language.

**DNA extraction and PCR of ITS region**

DNA from the ground samples was isolated and purified using a plant-DNA isolation kit (NucleoSpin Plant; Macherey-Nagel, Düren, Germany). The oligonucleotide primers for the amplification of the ITS region were synthesized by Genotec, Inc. (Daejeon, Korea). The universal primers that annealed at the 5’- and 3’-ends of the ITS region were ITS5F (5’-GGAAGTAAAAGTCGTAACAAGG-3’) and ITS4R (5’-TCCTCCGCTTATTGATATGC-3’), respectively [14]. PCR amplification was conducted in a 100-µl final reaction volume, and the reaction mixture consisted of 1.0 µM of each primer, 50 ng of extracted DNA, 0.2 mM of each deoxynucleoside triphosphate (dNTP), 1.5 mM MgCl₂, 10× reaction buffer (Biogen, Daejeon, Republic of Korea), and 1 unit of Taq DNA polymerase (Biogen). PCR was conducted for 30 cycles, with a thermal profile as follows: predenaturation at 94°C for 10 minutes, denaturation at 94°C for 30 seconds, primer annealing at 68°C for 30 seconds, and extension at 72°C for 30 seconds. The final cycle included a 10-minute extension at 72°C to ensure full extension of the products.

**Gel electrophoresis and DNA sequencing**

The PCR products were analyzed via electrophoresis of a 5-µl aliquot through a 1.0% (wt/vol) agarose gel (Caledon, Georgetown, ON, Canada) stained with ethidium bro-mide and visualized via UV transillumination. A 100-bp ladder (Bioneer, Daejeon, Republic of Korea) was employed as a size marker. The DNA from all samples was PCR-amplified and purified with a PCR-product purification kit (Suprec-02; Dakara, Korea), in accordance with the manufacturer’s instructions. The purified PCR products were sequenced by Genotec (Daejeon, Korea).

**ITS sequence comparison**

The DNA sequences obtained in the sequencing experiments were then used to conduct a comparison of the ITS regions. The entire sequence of the ITS1-5.8S-ITS2 was compiled using SeqMan software and the sequences were edited with the BioEdit software program [19]. The ITS sequences of the related Panax species were acquired from GenBank. Multiple alignments were conducted using the CLUSTAL X software program [20].

**Design of specific primers**

Two specific primers (quinR and ginR) for analyzing the mountain ginseng samples were designed on the basis of the SNP sites specific to the three mountain ginseng